
SYNOPSIS

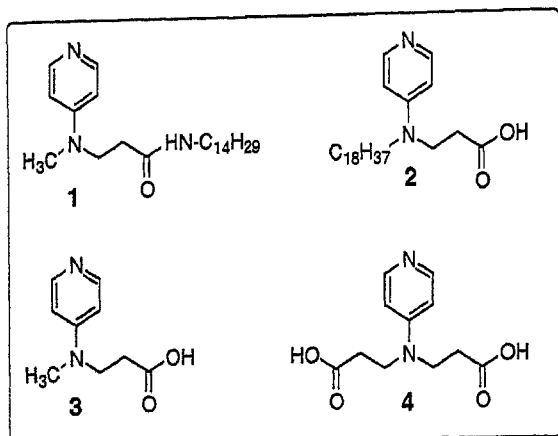
The thesis entitled “**Design and Synthesis of Novel Catalysts for the Hydrolysis of Organophosphates in Nanoaggregates: Experimental and Computational Studies**” illustrates the effectiveness of various cationic micellar and other nanoaggregates consisting of numerous novel molecular entities having covalently attached nucleophilic headgroups. These systems were employed as putative mimics of hydrolase enzymes. This thesis has been divided into seven chapters. Chapter 1 gives a general introduction about the area of micellar catalysis. Chapter 2 describes the effect of host micellar aggregates such as gemini micelles on rates of the hydrolysis reactions. In chapter 3, the 1-hydroxybenzotriazole based nucleophilic catalysts in cationic micelles. Chapter 4 explains the reactivity of 1-hydroxybenzotriazoles towards hydrolytic reactions by analyzing plausible electronic features of 1-hydroxybenzotriazole by *ab initio* and density functional theory. Chapter 5 and 6 deal with the design, synthesis and kinetics analysis of 1*H*-tetrazoles as efficient nucleophilic catalysts in cetyl trimethyl ammonium bromide (CTABr) micellar media. In these two chapters the design of efficient catalysts based on the quantum mechanical calculations has been shown. Finally, in Chapter 7, the design and synthesis of another class of 1*H*-tetrazole derivatives have been presented. These formed nanorods and nanowires in neutral aqueous media. Such organic 1D-nanomaterials exhibit efficient catalytic activity toward hydrolysis of organophosphates and alkanates.

Chapter 1. Design and Mimic of Hydrolase Enzymes: an Introduction.

Chapter 1 provides an introduction to the catalysis of hydrolytic reactions in various supramolecular aggregates. It gives a comprehensive account of the research work done by several research groups working in the area of supramolecular and bioorganic chemistry towards the design and development of enzyme mimics.

Chapter 2. Evidence of Enhanced Reactivity of DAAP nucleophiles toward Dephosphorylation and Deacylation Reactions in Cationic Micellar Media.

In chapter 2 the design and synthesis of 4,4'-(dialkylamino) pyridine (DAAP) based compounds, 1-4 have been presented. These nucleophiles catalytically cleave hydrophobic organophosphate and carboxylate esters in various host micellar aggregates at mildly alkaline pH. The role of the micellar reaction medium in such esterolytic reactions has been carefully examined in this work. Cationic ‘gemini’ surfactants (16-*m*-16, 2Br) based micellar aggregates provide more than an order of magnitude better reaction medium for the above reactions as

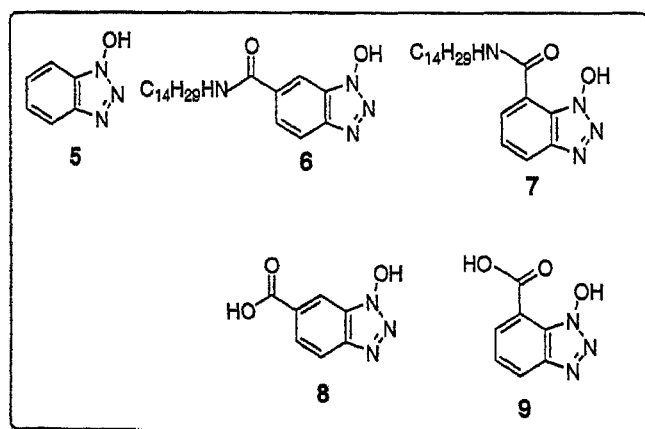


compared to micelles formed from their conventional single chain/charge counterpart, CTABr. The most rapid hydrolysis was observed with catalyst **2** for cleavage of *p*-nitrophenyl diphenyl phosphate (PNPDPP) and *p*-nitrophenyl hexanoate (PNPH), which can be attributed to its strong association with cationic host micelles via hydrophobic and ion-pairing

interactions. The catalytic turnover behavior of DAAP nucleophiles in the presence of excess substrates was also observed in both types of micellar media.

Chapter 3. Ester Cleavage Properties of Synthetic Hydroxybenzotriazoles in Cationic Monovalent and Gemini Surfactant Micelles.

In this chapter, the design and synthesis of four new hydroxybenzotriazole derivatives have been presented. Two of them, *N*-tetradecyl-1-hydroxy-1*H*-benzo[d][1,2,3]triazole-6-carboxamide (**6**) and *N*-tetradecyl-1-hydroxy-1*H*-benzo[d][1,2,3]triazole-7-carboxamide (**7**) possess long alkyl chain while the other two, 1-hydroxy-1*H*-benzo[d][1,2,3]triazole-6-carboxylic acid (**8**) and



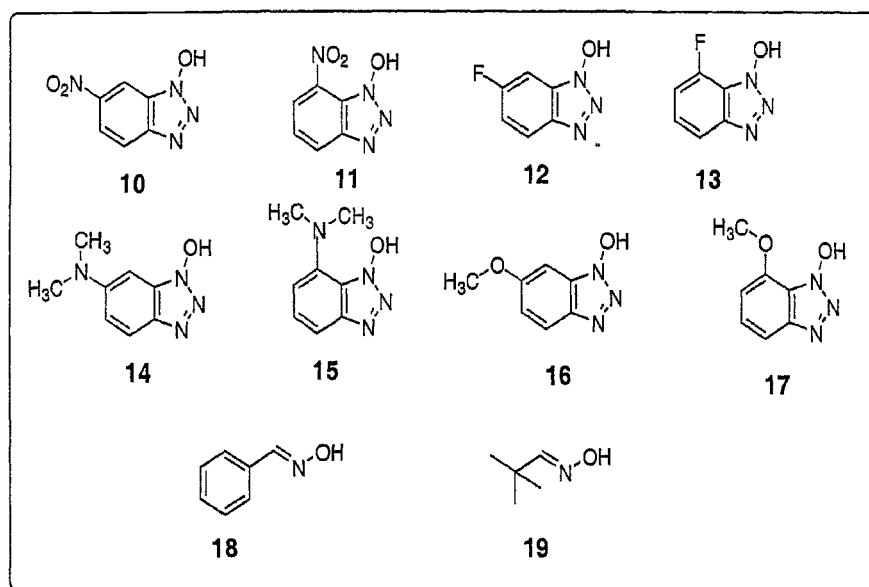
1-hydroxy-1*H*-benzo[d][1,2,3]triazole-7-carboxylic acid (**9**) have carboxylate side chains. These compounds along with their parent unsubstituted 1-hydroxybenzotriazole, (HOBt), **5**, have been examined for the cleavage of PNPH and PNPDPP in micelles with monovalent CTABr and the corresponding bis-cationic gemini

surfactants, 16-*m*-16, 2Br⁻ of identical chain length at 25 °C, pH 8.2. The apparent pK_a values of the HOBt derivatives in the micelles of CTABr or 16-4-16 gemini surfactants have been determined from the rate vs pH profiles and were found to be comparable. Catalytic system 8/16-4-16 shows over 2200 and 1650-fold rate enhancements in the hydrolysis of PNPDPP and PNPH respectively for identical reactions carried out at pH 8.2, 25 °C in buffered aqueous media. The

second-order rate constants for such bimolecular reactions were determined employing pseudophase micellar models. Experiments in which excess substrate were taken over HOBt derivatives demonstrated that the catalysts “turned over”, hydrolysis of the acylated or phosphorylated HOBt intermediates was rapid in either host micelles.

Chapter 4. Computational Study on Hydroxybenzotriazoles as Reagents for Ester Hydrolysis.

In this chapter, a detailed computational study on several derivatives of 1-hydroxybenzotriazole was presented. As shown in chapter 3, 1-hydroxybenzotriazole (**5**) and several of its derivatives

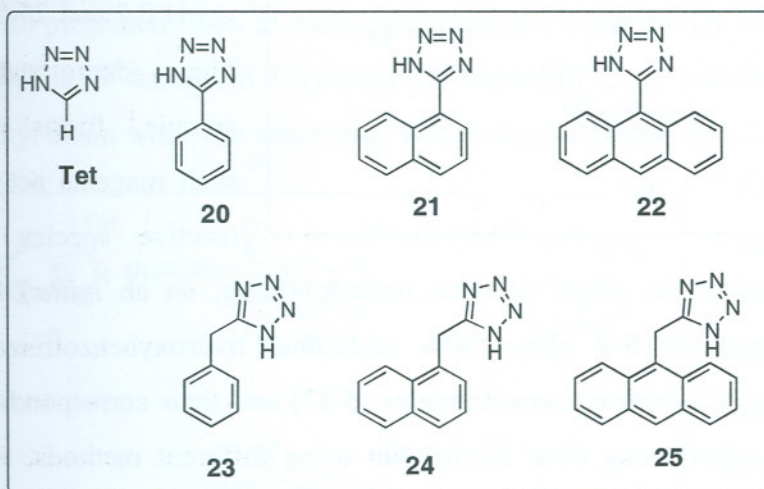


(**6-9**) demonstrate esterolytic activity toward activated esters such as PNPDP and PNPH in cationic micelles at pH 8.2 and 25 °C. The deprotonated anionic forms of such reagents act as reactive species in

the hydrolysis of ester. To rationalize the origin of their nucleophilicity, an *ab initio*/DFT computational study was performed on **5-9** along with additional hydroxybenzotriazole derivatives (**10-17**). The geometries of 1-hydroxybenzotriazoles (**5-17**) and their corresponding bases are discussed in detail. All calculations were carried out using different methods, *viz* restricted Hartree-Fock (RHF) and hybrid *ab initio*/DFT (B3LYP) using 6-31G* and 6-31+G* basis sets. Free energy of protonation ('fep') of the 1-hydroxybenzotriazoles (**5-17**), free energy of solvation ΔG_{aq} , and the corresponding $\text{p}K_{\text{a}}$'s have been calculated. Solvation free energies were calculated using density functional theory and the polarizable continuum model. In addition, to examine the reliability of calculated proton affinity, benzaldehyde oxime (**18**) and 2-methylpropionaldehyde oxime (**19**) (the experimental proton affinities of which are known) have been computed as reference systems using different methods and basis sets.

The experimental results indicate that the catalyst **8** is the most effective catalyst for the hydrolytic cleavages of PNPDP and PNPH. The calculated 'fep', pK_a and natural charge analysis results also predict same outcome. In general, the introduction of electron-withdrawing substituents on 1-hydroxybenzotriazoles facilitates the lowering of pK_a and 'fep'. As the pK_a values are lowered, greater percentage of such hydroxybenzotriazoles remains in their deprotonated, anionic forms at pH 8.2. Since the anionic forms are nucleophilic, pK_a lowering should enhance their ester cleaving capacity. However, such substitution also decreases the charge density on the catalytically active oxido atom (O_7). Taken these two factors together, it makes the derivatives only modestly better nucleophile in comparison to the parent 1-hydroxybenzotriazole. Interestingly, calculations indicated that the introduction of electron-donating groups does not significantly enhance the charge accumulation on the oxido atom (O_7) of 1-hydroxybenzotriazoles.

Chapter 5. Aromatic 1H-Tetrazole Derivatives in Cationic Micelles as Potent Reagents for Esterolysis at Neutral pH. Computational and Experimental Studies.



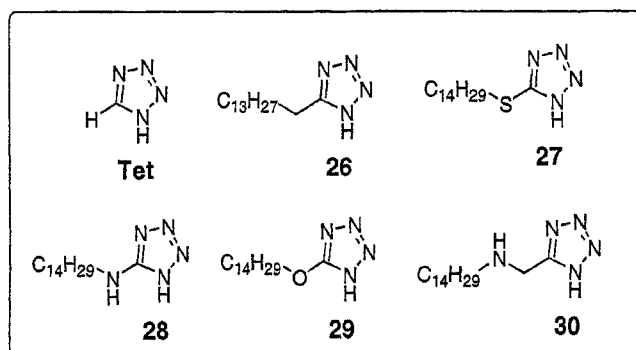
In this chapter the design, synthesis and kinetics of several 5-substituted tetrazole derivatives, **20-25** have been described. By performing a detailed *ab initio* computational study, it has been found that in 5-substituted 1H-tetrazole systems, introduction of an aromatic group at the 5-position of the tetrazole

ring reduces the charge on the tetrazole nucleus significantly. However, insertion of a methylene group between the tetrazole moiety and the aromatic ring raises the charge on the tetrazole nucleus considerably. All calculations have been performed using restricted Hartree-Fock (RHF) and hybrid *ab initio*/DFT (B3LYP) using 6-31G* and 6-31+G* basis sets. To estimate the nucleophilicity of these reagents the charges on the conjugate bases of each tetrazole derivatives were calculated using natural population (NBO) analysis both in gas-phase and in water. Attachment of aromatic residues with tetrazoles also enhances the hydrophobicity of the resulting

systems and this in turn rises their partitioning into micellar pseudophase. Notably, tetrazoles (**20-25**) also induced catalytic hydrolysis of hydrophobic organophosphate and carboxylate esters in cationic micelles at neutral pH when excess substrates were employed. To acquire better k_{obs} values for the cleavages of PNPDP and PNPH under micellar conditions, the hydrophobicity and charge on the N^- atom (nucleophile) of conjugate bases of tetrazoles are important. Higher hydrophobicity and large charge accumulation on N^- atom synergistically act together to make **25** as a very potent catalyst in this series of compounds. Impressive rate enhancements were observed with **25**/CTABr over background rates (CTABr alone) for the cleavages of PNPDP and PNPH at pH 7.0.

Chapter 6. Computational and Kinetic Studies on 5-substituted 1H-Tetrazoles as Potent Reagents for Ester Hydrolysis at Neutral pH.

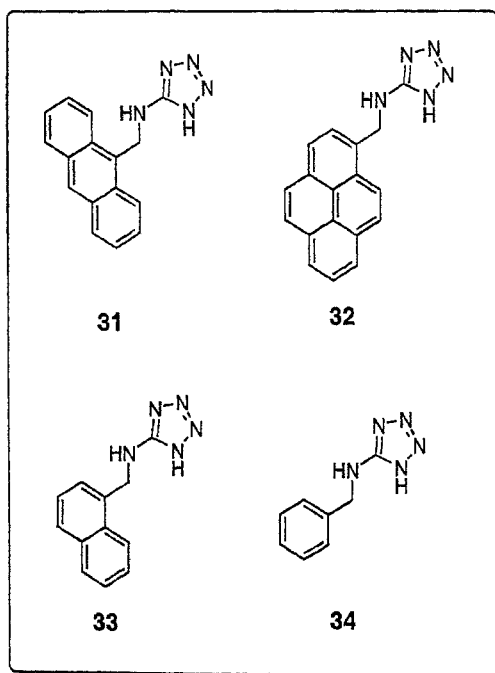
By performing a detailed *ab initio* computational study, it has been noticed that in 5-substituted 1H-tetrazole systems, introduction of a heteroatom e.g. N, O or S at the α -position of the tetrazole ring raises the charge on the tetrazole nucleus significantly. All calculations have been performed using restricted Hartree-Fock (RHF) and hybrid *ab initio*/DFT (B3LYP) methods employing 6-31G* and 6-31+G* basis sets. To estimate the nucleophilicity of



these reagents, the charges on conjugate bases of various tetrazole derivatives have been calculated using natural population (NBO) analysis in gas-phase and in water. Free energy of protonation (ΔG_{aq}) of the 1H-tetrazole derivatives (**Tet** and **26-30**), free energy of solvation, ΔG_{aq} , and the corresponding pK_a 's have been calculated. Since the calculation indicates that incorporation of heteroatom leads to enhanced nucleophilicity in their deprotonated anionic tetrazole forms, a series of 5-substituted 1H-tetrazole derivatives have been synthesized. These compounds indeed hydrolyze PNPDP and PNPH (in cationic CTABr micelles at pH 7.0 and 25 °C) with high efficiency. The pseudo-first-order rate constants (k_0) were determined for each catalyst for both substrates. The experimental and theoretical results showed that to achieve better k_{cat} values for the cleavage of PNPDP and PNPH under micellar conditions, charge on the N^- atom (nucleophile) of conjugate base is important. Replacing the α -CH₂ in hydrocarbon chain

with S (**27**), NH (**28**) or O (**29**), enhances the accumulation of charge on N⁻ in conjugate bases of these tetrazoles and subsequently increases inherent nucleophilic reactivity of above reagents in hydrolytic reactions. Significantly large rate enhancements were observed for the cleavage of PNPDP and PNPH at pH 7.0 in the presence of catalytic system **29**/CTABr over background (only CTABr). Tetrazole, **28** (α -isomer) showed 400-500% superior reactivity over **30** (β -isomer) under identical conditions. Natural charges obtained from NBO analysis (B3LYP/6-31+G*) are -0.94 and -0.852 on N⁻ in the conjugate bases of **29** and **30** respectively. This also predicts that **29** is a better nucleophile than **30**. All the newly synthesized tetrazole derivatives in micellar media displayed true catalytic properties by cleaving several fold excess of substrates.

Chapter 7. Synthesis and Esterolytic Properties of aqueous suspensions of Novel 1D-Organic Nanomaterials.



Chapter 7 deals with the preparation of novel organic nanoaggregates and their utility as efficient hydrolase mimics. One-dimensional organic nanomaterials such as nanorods and nanowires were prepared by simple reprecipitation method. DMSO solutions of 5(9-anthracenylmethylamino) 1H-tetrazole (**31**) and 5(1-pyrenylmethylamino) 1H-tetrazole (**32**) upon injection into water produce such nanoscale aggregates, which have been characterized by transmission electron microscopy, dynamic light scattering as well as absorption and emission spectroscopy. Nanorods (**31**) of length in the

range of couple of micrometers and diameters ranging 200 to 300 nanometers were observed. Nanowires of **32** lengths in the range of couple of micrometers and diameters of several tens of nanometers were observed. Thus, the aggregates had high aspect ratios. These nanomaterials were investigated for their ability to catalyze the hydrolysis of hydrophobic organophosphate and carboxylate esters at pH 7.0. They exhibit classical Michaelis-Menten kinetics. The parameters such as k_{obs} , K_M , V_{max} and k_{cat} were determined for the two types of nanoaggregates for both types

of substrates (PNPDPP and PNPH) At higher temperatures (25-80 °C) also these nanoaggregates were still intact and able to catalyze the hydrolytic reactions TEM, absorption and emission studies reveal that **33** or **34** have formed no aggregates in water They also do not catalyze the cleavage of either PNPDPP or PNPH This clearly suggests that an appropriate lipophile-hydrophile balance is essential for the formation of nanoaggregates and their consequent abilities to mediate catalytic esterolysis reactions at ambient pH